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# Association of Twin Zygosity With the Mean and Variance of Tooth Size

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To search for an association of twin zygosity with tooth size, 56 dental variables measured from 65 pairs of twins (43 MZ, 22 DZ) were studied. Results of the  $t'$  test for equality of the means showed no association of zygosity with any of the variables in males or in females. Results of the  $F'$  test for homogeneity of total variances between zygositys showed evidence for unequal total variances in 15 variables in males and 13 in females. Sex influence was further noted on the association of zygosity with the variance of tooth size. Where total variances were unequal, genetic variance estimates differed when only the within-pair mean squares were used and when combined estimates designed to be unbiased by differences in environmental variances were used.

**Key words:** Tooth size, Unequal total variance, Sex effect

## INTRODUCTION

The classical twin model that involves comparison of the within-pair differences in monozygotic (MZ) and dizygotic (DZ) twins has been used traditionally in genetic studies of dental traits, including tooth size. This procedure is based on the implied assumption that environmental variances of the two twin types are equal, where the term "environmental effects" encompasses all nongenetic effects such as cytoplasmic inheritance, maternal genotype, prenatal, postnatal, maternal, and developmental factors. There is increasing evidence that twins are subject to stresses that are unique to the twinning process itself, which may well constitute a further source of variance in twin data.

The critical test for equality of environmental variances of the two twin types is a test for homogeneity of total variances [2]. Strictly speaking, the MZ-DZ within-pair comparison cannot be applied if there is evidence that the total variances differ between zygositys. The documentation of the total variance of MZ and DZ twins in genetic and heritability estimates of tooth size is usually either ignored or concealed by the use of correlation coefficients.

Recently, Christian [4] reported that in the National Heart, Lung and Blood Institute (NHLBI) Collaborative Twin Study, 15 out of 31 quantitative traits had evidence for un-

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equal total variances in MZ and DZ twins. In addition, Reed et al [8] reported similar evidence for 21 out of 71 dermatoglyphic variables. Moreover, in several traits where significant genetic variation was obtained by comparing DZ-MZ within-pair variances and ignoring any heterogeneity of total variance, the genetic variation was no longer evident when an estimate unbiased by different environmental variances of MZ and DZ twins was used. It appears, then, that in twins, once thought to provide the strongest evidence for genetic influences on a number of biological variables, these influences may be obscured by environmentally caused differences in the total variances of MZ and DZ twins. It is therefore possible that the association of a twin type with quantitative dental traits may likewise be relatively common and certainly should be searched for.

Another level of association of twin type with a trait could be with the means. There are few data in the literature showing such associations. Havlik et al [5] reported that four of 11 clinical chemistry values showed evidence for inequality in MZ and DZ means in the NHLBI twin study. To our knowledge no previous dental twin studies had compared the means of the trait between zygositys.

The purpose of this study was to search for the association of twin zygosity with the mean and variance of tooth size.

## METHODOLOGY

Dental casts recorded from 65 pairs of Caucasian twins from the Indiana University twin panel were used as the sample; 32 pairs were male and 33 were female. Among the male twins, 18 were MZ and 14 were DZ pairs. Among the female twins, 25 were MZ and eight were DZ pairs. Zygosity was determined by serologic and dermatoglyphic data. Mesiodistal and buccolingual measurements were obtained from the dental casts for each of the 28 teeth of the secondary dentition, excluding the third molars, making a total of 56 tooth size variables that were studied. Measurements were obtained with a semiautomated electronic caliper system.

To determine if sex influences the association of twin zygosity with tooth size, all analyses were separately performed for males and females.

To test for equality between MZ and DZ twins in the means of each of the 56 variables studied, the  $t'$  test based on the nested (hierarchical) structure of twin data proposed by Christian and Norton [3] was used. In this mixed model, the analysis of variance involved a hierarchy consisting of zygositys (fixed effect), twin pairs within zygositys (random effect), and the two members within twin pairs (random effect), in that order. The statistical test comparing between zygositys therefore used only the among-pair mean squares of MZ and DZ twins as the error term, because the two members of a twin pair could not be considered independent of each other. Since the expected values of the mean squares among pairs are theoretically heterogeneous between zygositys [2], the "no pooling" procedure was used in calculating for the error term of the  $t'$  tests, and the degrees of freedom were approximated.

To test for homogeneity of total variances between zygositys, the  $F'$  test [2], comparing the sum of the mean squares within and among the DZ pairs with the corresponding sum for MZ pairs, was performed for each tooth size variable. First, the one-way analysis of variance was performed separately for the MZ and DZ sets to provide the mean squares. The larger sum of mean squares was used as the numerator of a two-tailed  $F'$  test and the probability level double that shown in the usual  $F$  tables. The numerator and the denominator degrees of freedom were approximated after Christian et al [2].

## RESULTS

Results of comparisons of means in Table 1 showed no association of the type of twinning with any of the 56 mean tooth size values.

Results of comparisons of total variances in Table 2 for males and in Table 3 for females demonstrated that, out of 56 tooth size variables, 15 in males and 13 in females have evidence for unequal total variances. Among male twins, eight variables were significant at the 0.05 probability level or less, and seven variables were significant at the 0.20 level or less. Among female twins, four variables were significant at the 0.05 probability level or less, and nine variables were significant at the 0.20 level or less. The 0.20 probability level was included because this  $F'$  test of total variance is a relatively insensitive test in the presence of genetic or environmental variance that is common to both MZ and DZ twins.

Tables 2 and 3 further suggest a sex influence in the pattern of association of twin zygosity with the variance in tooth size. Heterogeneity of total variance was exhibited primarily in the males among the upper anterior teeth and the lower posterior teeth, but in the females among the lower anterior teeth. In addition, all variables in males that showed unequal variances were not the same variables as in the females, except for the mesiodistal dimensions of the two lower first molars. Even in these two variables, males exhibited a larger total variance in the DZ twins, but the females exhibited a larger total variance in the MZ twins. Males showed a larger total variance in the DZ category for 14 out of the 15 specified variables where variance inequalities were detected. In the females, however, it was the MZ category that showed a larger total variance for all of the 13 variables in question.

In either sex, variables demonstrating heterogeneity in total variances were further explored to determine the source of such heterogeneity. The DZ and MZ sets were compared with respect to their among- and within-pair mean squares using a two-tailed  $F$  test. Results of comparisons are shown in Table 4. In the males, both the within-pair and among-pair mean squares were generally larger in the DZ twins. Results for females showed that most variables exhibited a larger DZ within-pair mean square than MZ twins and the among-pair mean square is larger in the MZ than in the DZ pairs, as would be expected if genetic variance were present.

To probe further the effect on genetic variance estimates of the association of twin zygosity with the variance when only the within-pair mean squares are used, the traditional within-pair  $F$  tests were computed for the variables in question, as shown in Table 5. Side by side are shown  $F'$  tests in the genetic analysis using the combined among-pair and within-pair estimate of the genetic variance, an estimate that is unbiased by significantly different environmental variances of MZ and DZ twins [2]. Both  $F$  and  $F'$  tests in Table 5 are one-tailed because the expected value of the numerator is greater than that of the denominator in the presence of genetic variance. It can be seen that in males, after correcting for bias in variance heterogeneity, genetic variance was no longer manifest, the reason being that the among-pair mean square was larger in the DZ than in the MZ twins. In females, the opposite was true in that the among-pair mean square was larger in the MZ than in the DZ twins, so that the combined estimate of genetic variance showed either the same or higher levels of significance. Some variables in females did show striking differences in genetic variance estimates between the two methods. Table 5 demonstrates clearly that in the presence of heterogeneity of the total variances, estimates of genetic variance were biased when the within-pair component was used alone, the direction of bias being dependent upon the relative magnitudes of the among-pair and within-pair mean squares in the MZ and DZ categories.

TABLE 1. *t* Value Testing Difference in Means Between MZ and DZ Twins\* (none of the *t* values is significant at 0.05 level)

	Male				Female			
	Upper		Lower		Upper		Lower	
	Right	Left	Right	Left	Right	Left	Right	Left
I1 MD	0.88	0.64	0.95	0.35	0.52	0.76	0.97	0.49
BL	0.32	0.20	0.83	0.98	0.44	0.07	0.21	0.50
I2 MD	0.49	0.09	0.59	0.32	0.56	0.89	1.18	1.62
BL	1.08	0.44	0.41	0.34	0.47	1.17	0.30	0.48
C MD	0.77	0.46	0.04	0.19	0.88	0.69	0.31	0.59
BL	0.07	0.34	0.17	0.21	0.75	0.99	0.70	0.35
P1 MD	0.38	0.67	0.63	0.30	0.18	0.62	0.65	0.07
BL	0.23	0.49	0.12	0.10	0.61	0.70	0.85	1.29
P2 MD	0.10	0.24	0.93	0.61	0.00	0.30	0.23	0.60
BL	0.30	0.17	1.12	0.61	0.00	0.63	0.38	0.27
M1 MD	0.02	0.39	0.54	0.40	0.44	1.10	0.33	0.09
BL	0.02	0.60	0.71	0.94	0.27	0.49	0.66	0.82
M2MD	1.21	1.10	0.40	0.43	0.01	1.40	0.82	0.39
BL	0.04	0.07	0.60	0.13	0.10	0.48	0.45	0.52

\*I1 and I2 = central and lateral incisors; C = canine; P1 and P2 = first and second premolars; M1 and M2 = first and second molars; MD = mesiodistal dimension; BL = buccolingual dimension.

## DISCUSSION

If there is an association between the type of twinning and a trait being studied, the estimate of genetic variance for this trait may well be biased. Such association could become manifest in inequality either of the means or of the variances between zygositys.

If the means differ between zygositys, further genetic analysis using MZ and DZ twins is unwarranted, one reason being that genetic variances may not be equal between the twin types. No differences in the means of tooth size were found between MZ and DZ twins, so that we may assume that, for each variable, the two twin types belong to distributions with the same location along the scale and we may proceed with further variance analysis.

In spite of the small sample size, our results have indicated that the assumption of homogeneity of total variances may not be tenable for approximately one-fourth of the tooth size variables studied in either males or females, more than what we would expect from chance occurrence alone. Generally, a large number of twins is necessary to detect a difference because of the relatively low sensitivity of the  $F'$  test used. It is therefore possible that with a larger twin sample more or different tooth size variables may further be detected to exhibit total variance heterogeneity.

In the relatively common situation where MZ and DZ group means do not differ significantly but the total variances exhibit heterogeneity, the most likely cause is unequal environmental influences in the two types of twins [6]. These results therefore constitute evidence that a most basic assumption of environmental variance equality in human twin studies of tooth size is not tenable and that available genetic variance and heritability estimates for tooth size are biased.

The heterogeneity of total variances between MZ and DZ twins is observed to be concentrated on the mesiodistal dimensions of the upper anterior teeth in males and on the same dimensions but of the lower anterior teeth in females, suggesting a possible sex influence on the association of twin type with the total variance. This finding is further strengthened by comparing among-pair as well as within-pair variances between MZ and DZ twins. Both males and females generally show larger DZ than MZ within-pair mean squares, as expected, but the sexes differ markedly with respect to the relative magnitudes of the among-pair mean squares between MZ and DZ twins. Sex influences, therefore, tend to confound the variance heterogeneity of these tooth size variables to bias seriously results of twin studies where sex effects have not been accounted for. This kind of bias is further demonstrated in Table 5. Where total variances are unequal, genetic variance estimates differ markedly when within-pair variance estimates are compared with the estimates combining within- and among-pair mean squares.

Two important implications in twin studies of tooth size arise from these results. First, among-pair sex differences should be accounted for. Second, preliminary tests of means and total variances should be performed. These data preliminarily indicate the possibility of different environmental influences on tooth size development between zygositys. Boklage et al [1] and Potter and Boklage [7] have shown multivariate differences between zygositys regarding symmetry development as well as differences in the within-individual correlations for the same dental variables. Further study of these differences may lead to important discoveries of factors determining tooth size.

TABLE 2. *F'* Tests of Total Variance Homogeneity Between Male MZ and DZ Twins\*

	AMS <sup>a</sup> + WMS <sup>b</sup>				AMS <sup>a</sup> + WMS <sup>b</sup>				F'	P <sup>c</sup>
	Upper right		Upper left		Upper right		Upper left			
	MZ	DZ	F'	P <sup>c</sup>	MZ	DZ	F'	P <sup>c</sup>		
I1 MD	0.3852	1.1703	3.04	< 0.05 (DZ)	0.4635	0.9986	2.15	< 0.20 (DZ)		
BL	0.5616	0.5659	1.01		0.6905	0.6380	1.08			
I2 MD	0.5750	1.0964	1.91	< 0.20 (DZ)	0.6512	0.9105	1.40	< 0.20 (DZ)		
BL	0.6466	1.1479	1.78	< 0.20 (DZ)	0.5822	1.1397	1.96	< 0.05 (DZ)		
C MD	0.1546	0.5249	3.40	< 0.05 (DZ)	0.1709	0.4833	2.83	< 0.05 (DZ)		
BL	0.8011	0.7784	1.03		0.7704	1.3225	1.72			
P1 MD	0.2105	0.2541	1.21		0.2386	0.2791	1.17			
BL	0.3333	0.4480	1.34		0.4585	0.3923	1.17			
P2 MD	0.2436	0.3017	1.24		0.2675	0.3820	1.43			
BL	0.3610	0.4701	1.30		0.3312	0.5262	1.59			
M1 MD	0.2854	0.7022	2.46	< 0.05 (DZ)	0.3261	0.6663	2.04	< 0.20 (DZ)		
BL	0.5513	0.7503	1.36		0.4611	0.6973	1.51			
M2 MD	0.8026	0.6202	1.29		0.7546	0.7944	1.05			
BL	1.0726	1.3837	1.29		1.0309	1.3058	1.27			



TABLE 3. *F'* Tests of Total Variance Homogeneity Between Female MZ and DZ Twins\*

	AMS <sup>a</sup> + WMS <sup>b</sup>			AMS <sup>a</sup> + WMS <sup>b</sup>			p <sup>c</sup>
	MZ	DZ	F'	MZ	DZ	F'	
	Upper right			Upper left			
I1 MD	0.8755	0.6527	1.34	0.8816	0.6519	1.35	
BL	0.5452	0.5956	1.09	0.5904	0.8332	1.41	
I2 MD	0.8931	0.9068	1.02	0.8322	0.4821	1.73	
BL	0.9727	0.8492	1.15	1.0673	0.6165	1.73	
C MD	0.3862	0.2215	1.74	0.3884	0.2080	1.87	
BL	0.5018	0.5917	1.18	0.5943	0.8664	1.46	
P1 MD	0.5476	0.1948	2.81	0.5578	0.3813	1.46	
BL	0.5022	0.7884	1.57	0.4306	0.5771	1.34	
P2 MD	0.5858	0.3886	1.51	0.6788	0.4721	1.44	
BL	0.6635	0.2772	2.39	0.7139	0.3965	1.80	
M1 MD	0.5279	0.4069	1.30	0.4244	0.3257	1.30	
BL	0.6508	0.4884	1.33	0.6493	0.7032	1.08	
M2 MD	0.6052	0.6011	1.01	0.5908	0.2033	2.91	< 0.20 (MZ)
BL	0.8727	0.9243	1.06	0.8774	1.1359	1.29	





TABLE 4. F Tests of Equality Between MZ and DZ Twins With Respect to Among-Pair Mean Squares and Within-Pair Mean Squares for Those Variables Exhibiting Heterogeneity of Total Variances\*

	Among-pair MS				Within-pair MS			
	MZ	DZ	F	p <sup>a</sup>	MZ	DZ	F	p <sup>a</sup>
<b>Male</b>								
Upper right								
I1 MD	0.3505	0.8603	2.45	< 0.10 (DZ)	0.0347	0.3100	8.93	< 0.002 (DZ)
I2 MD	0.5400	0.8396	1.55	(DZ)	0.0350	0.2568	7.34	< 0.002 (DZ)
I2 BL	0.4685	0.9336	1.99	< 0.20 (DZ)	0.1781	0.2143	1.20	(DZ)
C MD	0.1393	0.3649	2.62	< 0.10 (DZ)	0.0153	0.1600	10.46	< 0.002 (DZ)
M1MD	0.2496	0.5611	2.25	< 0.20 (DZ)	0.0358	0.1411	3.94	< 0.01 (DZ)
Upper left								
I1 MD	0.4199	0.7332	1.75	(DZ)	0.0436	0.2654	6.09	< 0.002 (DZ)
I2 BL	0.5066	0.9543	1.88	(DZ)	0.0756	0.1854	2.45	< 0.10 (DZ)
C MD	0.1276	0.3194	2.50	< 0.10 (DZ)	0.0433	0.1639	3.79	< 0.01 (DZ)
M1MD	0.2675	0.5713	2.14	< 0.20 (DZ)	0.0586	0.0950	1.62	(DZ)
Lower right								
P2 MD	1.0325	0.2391	4.32	< 0.02 (MZ)	0.0433	0.1064	2.46	< 0.10 (DZ)
M1MD	0.5526	1.2613	2.28	< 0.20 (DZ)	0.0619	0.2386	3.85	< 0.01 (DZ)
M1BL	0.2078	0.6599	3.18	< 0.05 (DZ)	0.0706	0.0936	1.33	(DZ)

Lower left													
P2 BL	0.2753	0.5957	2.16	< 0.20 (DZ)	0.0628	0.2289	3.64	< 0.02 (DZ)					
M1MD	0.6239	1.1396	1.83	(DZ)	0.0406	0.2100	5.17	< 0.002 (DZ)					
M1BL	0.2852	0.7651	2.68	< 0.10 (DZ)	0.0644	0.0939	1.46	(DZ)					
Female													
Upper right													
P1 MD	0.5090	0.0942	5.40	< 0.05 (MZ)	0.0386	0.1006	2.61	< 0.10 (DZ)					
P2 BL	0.6271	0.1728	3.63	< 0.10 (MZ)	0.0364	0.1044	2.87	< 0.05 (DZ)					
Upper left													
M2MD	0.4620	0.1696	2.72	< 0.20 (MZ)	0.1288	0.0337	3.82	< 0.10 (MZ)					
Lower right													
I1 MD	0.4183	0.1471	2.84	< 0.20 (MZ)	0.0266	0.0519	1.95	< 0.20 (DZ)					
I1 BL	0.4290	0.0539	7.96	< 0.01 (MZ)	0.0618	0.1625	2.63	< 0.10 (DZ)					
I2 MD	0.3185	0.0299	10.65	< 0.01 (MZ)	0.0276	0.0169	1.63	(MZ)					
I2 BL	0.5153	0.1042	4.95	< 0.05 (MZ)	0.0428	0.1306	3.05	< 0.05 (DZ)					
M1MD	0.8185	0.3185	2.57	0.20 (MZ)	0.1128	0.1444	1.28	(DZ)					
Lower left													
I1 MD	0.4096	0.1063	3.85	< 0.10 (MZ)	0.0244	0.0694	2.84	< 0.05 (DZ)					
I2 MD	0.3664	0.0225	16.28	< 0.002 (MZ)	0.0592	0.0700	1.18	(DZ)					
I2 BL	0.5938	0.1135	5.23	< 0.05 (MZ)	0.0502	0.1031	2.05	< 0.20 (DZ)					
C MD	0.2446	0.0942	2.60	< 0.20 (MZ)	0.0222	0.0294	1.32	(DZ)					
M1MD	0.7310	0.2842	2.57	0.20 (MZ)	0.1154	0.0419	2.75	0.20 (MZ)					

\*I1 and I2 = central and lateral incisors; C = canine; P1 and P2 = first and second premolars; M1 and M2 = first and second molars; MD = mesiodistal dimension; BL = buccolingual dimension.

<sup>a</sup>All F tests are two-tailed tests. The twin type providing the numerator (larger) mean square is shown with the P level.

TABLE 5. One-Tailed F Tests for Presence of Genetic Variance Using Within-Pair Mean Squares Alone (F) and Combined With Among-Pair Mean Squares (F'), for Those Variables Exhibiting Heterogeneity of Total Variances\*

		Within-pair estimate		Combined estimate	
		F <sup>a</sup>	p <sup>b</sup>	F' <sup>c</sup>	p <sup>b</sup>
Male					
Upper right	I1 MD	8.93	< 0.001	0.74	
	I2 MD	7.34	< 0.001	0.91	
	I2 BL	1.20		0.61	
	C MD	10.46	< 0.001	0.79	
	M1 MD	3.94	< 0.005	0.65	
Upper left	I1 MD	6.09	< 0.001	0.88	
	I2 BL	2.45	< 0.05	0.67	
	C MD	3.79	< 0.005	0.80	
Lower right	M1 MD	1.62		0.58	
	P2 MD	2.46	< 0.05	4.03	< 0.005
	M1 MD	3.85	< 0.005	0.60	
Lower left	M1 BL	1.33		0.41	
	P2 BL	3.64	< 0.01	0.77	
	M1 MD	5.17	< 0.001	0.71	
	M1 BL	1.46		0.46	
Female					
Upper right	P1 MD	2.61	< 0.05	4.59	< 0.005
	P2 BL	2.87	< 0.025	3.50	< 0.025
Upper left	M2 MD	0.26		1.66	
Lower right	I1 MD	1.95		2.71	< 0.05
	I1 BL	2.63	< 0.05	5.11	< 0.001
	I2 MD	0.61		5.83	< 0.001
	I2 BL	3.05	< 0.025	4.39	< 0.005
	M1 MD	1.28		2.23	
Lower left	I1 MD	2.84	< 0.025	3.67	< 0.025
	I2 MD	1.18		5.34	< 0.001
	I2 BL	2.05		4.26	< 0.005
	C MD	1.32		2.35	
	M1 MD	0.36		1.93	

\*See Table 4 for mean square values. I1 and I2 = central and lateral incisors; C = canine; P1 and P2 = first and second premolars; M1 and M2 = first and second molars; MD = mesiodistal dimension, BL = buccolingual dimension.

<sup>a</sup>F = Within-DZ mean square / Within-MZ mean square.

<sup>b</sup>Probability level of 0.05 or less is used for both F and F' tests.

<sup>c</sup>F' = (Among-MZ mean square + Within-DZ mean square) / (Among-DZ mean square + Within-MZ mean square).

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